PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Kai Wang et al.
Application No.	:	09/801,196
Filed	:	March 6, 2001
For	:	A NOVEL MATRIX METALLOPROTEINASE (MMP-25) EXPRESSED IN SKIN CELLS
Examiner	:	Jehanne E. Souaya
Art Unit	:	1634
Docket No.	:	240083.509

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.131

Assistant Commissioner:

We, Kai Wang, Ryan Smith, Mark Fajardo (now deceased) *by* Randall C. Schatzman, a duly authorized Officer of Celltech R & D. Inc., which is a successor in interest to Chiroscience R & D, Inc., and Patrick Moss, do hereby declare the following.

1. We are coinventors of the subject matter that is described and claimed in the above-identified patent application ("subject application").

2. All of the work described within this Declaration and in the attached Exhibit A was performed in the United States by ourselves or on our behalf and under our direction.

3. We have reviewed our records, including the Invention Disclosure submitted herewith (*see* attached Exhibit A), and readily conclude that methods and compounds, as claimed in the subject application, were conceived prior to March 26, 1999, the filing date of U.S. Patent No. 6,331,427 issued to Robison. Further, due diligence was exercised from this time period until the invention was either actually reduced to practice or until the filing of U.S. Provisional Application No. 60/187,196, filed March 6, 2000, from which the subject application claims the benefit of priority.

4. Prior to March 26, 1999, we conceived the invention described in the subject application, that is, an isolated nucleic acid molecule consisting of a nucleotide sequence set forth in SEQ ID NO:1; a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:5; to a nucleic acid molecule that is at least 85% identical to the nucleotide sequence set forth in SEQ ID NO:5, wherein the nucleic acid molecule encodes a MMP-25 polypeptide that exhibits the same relative proteolytic activity as a wild-type MMP-25 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:6, or the complementary sequence thereof; and to related compositions and methods.

5. The following Exhibit A (annexed hereto) represents an Invention Disclosure prepared and kept in the regular course of business at Celltech R & D, Inc. (successor in interest to Chirosciences R & D, Inc.). The dates have been removed from the copies submitted herewith. Our understanding, based on discussions with Applicants' representatives, is that this is a permissible Patent Office practice.

6. Exhibit A, which is a photocopy of the Invention Disclosure, discloses that prior to March 26, 1999, we conceived the invention described in the subject application. We discovered in a cDNA library a polynucleotide fragment (SEQ ID NO:1 of the subject application) that encoded a polypeptide, which database searches indicated was a novel member of the MMP family (*see* Exhibit A, page 7). We then discovered the full length MMP-25 nucleic acid molecule having the sequence set forth in SEQ ID NO:5 of the subject application, and we determined the coding sequence for the MMP-25 polypeptide, which has the amino acid sequence set forth in SEQ ID NO:6 of the subject application (Exhibit A, page 7; Figure 1). We deduced therefrom that the encoded MMP-25 polypeptide contains conserved structural features that are characteristic of members of the matrix metalloproteinase family, including a signal peptide, a zinc-dependent binding domain, and a hemopexin domain. In addition, and also as found in other MMP polypeptides, the cystein-switch sequence is located within the pro-peptide (*see* Exhibit A, page 7; Figures 1 and 2). We also discovered a splice variant nucleic acid molecule having the sequence set forth in SEQ ID NO:3 that encodes the polypeptide set forth in SEQ ID NO:4 of the subject application, which lacks the zinc/calcium binding domain (*see* Exhibit A, page 8 and Figure 2). We then identified the genomic location of MMP-25-encoding sequences (*see* Exhibit A, page 8-9) and determined that MMP-25-encoding polynucleotides (SEQ ID NOs:3 and 5) were expressed in fetal skin (*see* Exhibit A, page 8; Figure 3).

7. In summary, upon review of the Invention Disclosure (Exhibit A), of which the above-cited pages are representative, we have concluded that, at least prior to March 26, 1999, we had conceived of the methods and compounds as described and claimed within the subject application. Furthermore, our conception of the invention led to further research, diligently undertaken, resulting in an actual reduction to practice or in the filing of the subject application.

8. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

10/23/03
Date

Kai Wang

10/22/03
Date

Ryan Smith

10/22/03
Date

Mark Fajardo (now deceased)

By Randall C. Schatzman, a duly authorized Officer of Celltech R & D, Inc., which is a successor in interest to Chiroscience R & D, Inc. pursuant to the attached employment agreement at paragraph 5 (Exhibit B)

10/22/03
Date

Patrick Moss

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CONSENT TO ASSIGNMENT OF AND AGREEMENT TO AMEND INVENTION AND PROPRIETARY INFORMATION AGREEMENT

Whereas, the undersigned employee ("Employee") entered into an Invention and Proprietary Information Agreement (the "Agreement") in connection with Employee's employment by Darwin Molecular Corporation, a Delaware corporation ("Darwin"), a copy of which is attached hereto as Exhibit A;

Whereas, as part of a corporate reorganization, effective March 1, 1998 Darwin established a wholly-owned subsidiary known as Chiroscience R & D, Inc., a Delaware Corporation ("Chiroscience"), whose purposes include conducting certain of the business operations previously undertaken by Darwin;

Whereas, effective March 1, 1998, as part of the foregoing-described corporate reorganization, certain assets and liabilities of Darwin were transferred to Chiroscience, including all rights and obligations arising out of the Agreement and the employment relationship between Employee and Darwin;

Whereas, paragraph 10 of the Agreement expressly provides that the Agreement shall be for the benefit of Darwin's successors and assigns; and

Whereas, Employee and Chiroscience desire to continue the employment relationship first begun between Employee and Darwin with Chiroscience acting as the successor employer to Darwin effective March 1, 1998.

Now, therefore, Employee and Chiroscience hereby agree, pursuant to this Consent to Assignment of and Agreement to Amend Invention and Proprietary Information Agreement (this "Amended Agreement") as follows:

1. All capitalized terms not otherwise defined herein, shall have the same meanings as defined in the Agreement.

2. Employee acknowledges that Employee has been notified of and, to the extent required by law, does hereby consent to the assignment as of March 1, 1998 by Darwin to Chiroscience of all rights, duties and obligations of Darwin arising under the Agreement.

3. Employee acknowledges that Employee has been notified of and, to the extent required by law, does hereby consent to the assignment by Darwin to Chiroscience of all rights, duties and obligations of Darwin arising out of the employment relationship between Employee and Darwin as it existed on March 1, 1998.

4. Employee and Chiroscience agree that effective March 1, 1998, the Agreement is amended as follows:

Wherever Darwin Molecular Corporation appears in the Agreement, it shall be replaced by Chiroscience R & D, Inc., a Delaware corporation, and the term "Company" as used in the Agreement shall be interpreted to mean Chiroscience R & D, Inc., a Delaware corporation.

5. Employee and Chiroscience agree that effective March 1, 1998, paragraph 4 of the Agreement is deleted in its entirety and replaced as follows:

4. During or after my employment, upon the Company's request and at the Company's expense, I will execute all papers in a timely manner and do all acts necessary to apply for, secure, maintain, defend or enforce patents, copyrights and any other legal rights in the United States and foreign countries in Inventions and Proprietary Information covered by Paragraphs 2 and 3, and I will execute all papers and do any and all acts necessary to assign and transfer to the Company or any person or party to whom the Company is obligated to assign its rights, my entire right, title and interest in and to such Inventions and Proprietary Information. I hereby irrevocably designate and appoint the Company and its duly authorized officers and agents as my agent and attorney-in-fact to act for me, and in my behalf, to execute and process any such papers and to do all other lawful acts to further the intent of this Paragraph 4. Effective March 1, 1998, I hereby assign and transfer to Darwin Discovery Limited, a corporation organized and existing under the laws of England, a member of the Chiroscience Group of companies, and an affiliate of the Company, my entire right, title and interest in and to all Inventions and Proprietary Information.

6. Employee hereby represents that all representations made in connection with the Agreement, including those made pursuant to paragraph 5 of the Agreement, are true and accurate as of the effective date of this Amended Agreement.

7. Except as otherwise expressly agreed herein, the Agreement shall remain in full force and effect.

I HAVE READ AND FULLY UNDERSTAND THE FOREGOING.

Employee _____

Mark A. Fajardo

Print Name: _____

12/21/98

Date

Chiroscience R & D, Inc.

By: _____

Name: Linda J. Nyari

Title: Vice President & General Councel

Date: 04/28/99

DARWIN MOLECULAR CORPORATION

INVENTION AND PROPRIETARY INFORMATION AGREEMENT

In an effort to define and clarify my rights and obligations as an employee and the rights and obligations of Darwin Molecular Corporation and any of its subsidiaries and affiliates to which its employees are assigned (the "Company") and MARK A. FAJARDO

In recognition of the importance of confidential information, trade secrets and inventions to the Company and

In consideration of my employment by the Company, any opportunities for advancement or reassignment that the Company may from time to time offer me, the compensation paid to me in connection with such employment and any stock and/or stock options which have been or may be granted to me by the Company,

I agree as follows:

1. For purposes of this Agreement the terms:

   (a) "Inventions" means discoveries, developments, designs, improvements, inventions and works of authorship, whether or not patentable, copyrightable or otherwise legally protectable. This includes, but is not limited to, any new machine, article of manufacture, biological material, method, process, technique, use, equipment, device, apparatus, system, compound, formulation, composition of matter, design or configuration of any kind, or any improvement thereon and

   (b) "Proprietary Information" means information and materials (biological, chemical or otherwise) not generally known or available outside the Company and information and materials entrusted to the Company by third parties. This includes, but is not limited to, trade secrets, confidential knowledge, ideas, source and object codes, biological materials such as nucleic acids, proteins, organisms, cell lines, antibodies or antigen source materials, or fragments thereof, chemical materials such as compounds or reagents, information about chemical or biological materials such as structural formulae or processes creating, utilizing or otherwise involving such materials and information which may relate, for example, to Inventions, research, development, manufacturing, business plans, personnel, purchasing, financial data, marketing or selling. Proprietary Information may include or may be contained in material such as drawings, samples, prototypes, data, procedures, specifications, reports, studies, customer or supplier lists, budgets, cost or price lists, compilations or computer programs, or may be in the nature of unwritten knowledge or know-how.

2. All Proprietary Information which is made available to me or which I conceive, create, develop, reduce to practice, or compile, either alone or with others, during the term of my employment shall be the exclusive property of the Company. I will preserve in confidence and will not disclose or use, either during or after the term of my employment, any Proprietary Information, except as required in my work for the Company or as authorized in writing by the Company. With respect to Proprietary

Information received by the Company from a third party, I will abide by any additional terms and conditions (including limitations on use) imposed upon the Company by the third party of which I am aware. Upon termination of my employment or upon request, I will deliver to the Company all forms of materials in my possession which contain or embody any Proprietary Information. In my work for the Company, I will refrain from unauthorized use of information belonging to my former employers or other third parties.

3. I hereby assign to the Company my entire right, title and interest in and to all Inventions which I conceive, create, develop or reduce to practice, either alone or with others, during the term of my employment. I will promptly and fully disclose to the Company any such Inventions.

NOTICE REQUIRED BY REVISED CODE OF WASHINGTON 49.44.140: Any assignment of Inventions required by this Agreement does not apply to an Invention for which no equipment, supplies, facility or trade secret information of the Company was used and which was developed entirely on the employee's own time, unless (a) the Invention relates (i) directly to the business of the Company or (ii) to the Company's actual or demonstrably anticipated research or development or (b) the Invention results from any work performed by the employee for the Company.

4. During or after my employment, upon the Company's request and at the Company's expense, I will execute all papers in a timely manner and do all acts necessary to apply for, secure, maintain, defend or enforce patents, copyrights and any other legal rights in the United States and foreign countries in Inventions and Proprietary Information covered by Paragraphs 2 and 3, and I will execute all papers and do any and all acts necessary to assign and transfer to the Company or any person or party to whom the Company is obligated to assign its rights, my entire right, title and interest in and to such Inventions and Proprietary Information. I hereby irrevocably designate and appoint the Company and its duly authorized officers and agents as my agent and attorney-in-fact to act for me, and in my behalf, to execute and process any such papers and to do all other lawful acts to further the intent of this Paragraph 4.

5. I have prepared and attached hereto a list of all Inventions, patent applications and patents conceived, created, developed or reduced to practice by me or with others prior to my employment with the Company, which are subject to prior agreements or which I desire to exclude from this Agreement, or if no such list is attached, I hereby represent and warrant that there are no such Inventions, patent applications or patents. If in the course of my employment with the Company, I use or incorporate into a product or process an Invention not covered by Paragraph 3 of this Agreement in which I have an interest, the Company is hereby granted an exclusive, fully paid-up, royalty-free, perpetual, worldwide license of my interest (with right to sublicense) to make, have made, use and sell such Invention without restriction.

6. During the term of my employment and for one (1) year thereafter, I will not, for my benefit or the benefit of others without the Company's written consent (a) engage in research or development with respect to the same or similar projects on which I was performing research or development for the Company or (b) directly or indirectly be employed or involved with any business unit developing or exploiting any products or services that are competitive with products or services (i) being developed or exploited by the Company during my employment and (ii) on which I worked or about which I learned Proprietary Information during my employment with the Company.

7. During the term of my employment and for one (1) year thereafter, I will not personally or through others recruit, solicit or induce in any way any employee, advisor or consultant of the Company to terminate his or her relationship with the Company or to engage in activities competitive with the Company.

8. I acknowledge that any violation of this Agreement by me will cause irreparable injury to the Company and I agree that the Company will be entitled to extraordinary relief in court, including, but not limited to, temporary restraining orders, preliminary injunctions and permanent injunctions without the necessity of posting a bond or other security and without prejudice to any other rights and remedies that the Company may have for a breach of this Agreement.

9. I agree and understand that nothing in this Agreement will confer any right with respect to continuation of my employment by the Company, nor will it interfere with the Company's right to terminate my employment at any time.

10. The obligations of this Agreement will continue beyond the termination of my employment and will be binding on my heirs, assigns and legal representatives. If any obligation herein is held to be too broad to be enforced, it will be construed to be enforceable only to the full extent permitted by law. This Agreement is for the benefit of the Company, its successors and assigns (including all present and future subsidiaries, affiliates, joint ventures and associated companies) and is not conditioned on my employment for any period of time or compensation therefor. This Agreement will be governed by and construed in accordance with the laws of the state of Washington (regardless of its choice-of-law provisions).

I HAVE READ AND FULLY UNDERSTOOD THIS AGREEMENT.

Mark A. Fajardo
Signature of Employee

MARK A. FAJARDO
Name of Employee (Please Print)

1-9-97
Date

Inventions listed on attached: ✓ Yes ___ No

12/1/98

Lily Okimoto
Signature of Witness for
Darwin Molecular Corporation

Jennifer Okimoto
Name of Witness (Please Print)

AUG-20-2003 11:55 CELLTECH R D, INC. INC

To my knowledge
I am not a part
of any inventions that
have been patented.
Mark C. Fujiuchi
1/97

DARWIN MOLECULAR

Patent Information Requested

Patent Title _____

_____

Inventors/Co-Inventors _____

_____

Where was the work done? _____

Who filed the patent? _____

Has this patent been issued? _____

What date was this patent issued? _____

What is this patent's number? _____

Patent Title _____

_____

Inventors/Co-Inventors _____

_____

Where was the work done? _____

Who filed the patent? _____

Has this patent been issued? _____

What date was this patent issued? _____

What is this patent's number? _____

CHIROSCIENCE

CHIROSCIENCE R&D, INC.

DOCUMENT NO: 99-002

INVENTION DISCLOSURE FORM

PLEASE USE THIS FORM FOR SUBMITTING AN INITIAL DISCLOSURE OF YOUR INVENTION. IF NECESSARY, PLEASE ATTACH AND NUMBER ANY ADDITIONAL PAGES. AFTER COMPLETING PLEASE HAVE YOUR DIRECTOR SIGN, THEN FORWARD TO THE LEGAL DEPARTMENT.

I. DESCRIPTIVE TITLE OF THE INVENTION:

A novel matrix metalloproteinase

II. INDIVIDUAL SUBMITTING THIS FORM

FULL NAME: Kai Wang

HOME ADDRESS (STREET): 5204 Somerset Dr. SE

CITY, COUNTY, STATE, ZIP: Bellevue, King, Washington 98006

WORK PHONE: 425-489-8046

CITIZENSHIP: US

SIGNATURE: Kai Wang

DATE: _____

III. OTHER INDIVIDUALS WHO ASSISTED IN DEVELOPING THIS INVENTION		
FULL NAME:	FULL NAME:	FULL NAME:
Ryan Smith	Mark Fajardo	PATRICK MOSS
HOME ADDRESS (STREET):	HOME ADDRESS (STREET):	HOME ADDRESS (STREET):
3223 NW 67th St	1124 N.E. 147th St.	1850 N 163rd St
CITY, COUNTY, STATE, ZIP:	CITY, COUNTY, STATE, ZIP:	CITY, COUNTY, STATE, ZIP:
Seattle, King, WA 98117	Shorline, King, WA 98155	Shorline, King, WA 98133
WORK PHONE:	WORK PHONE:	WORK PHONE:
425-489-8107	425-489-8011	425-489-8093
CITIZENSHIP:	CITIZENSHIP:	CITIZENSHIP:
US	US	US
SIGNATURE:	SIGNATURE:	SIGNATURE:
DATE:	DATE:	DATE:

IV. PLANNED OR ACTUAL USE OF THE INVENTION:

1. Treatment of an individual in such a way as to enhance or to inhibit MMP 25 polypeptide activity by administration of a therapeutically effective amount of an agonist or antagonist that directly interacts with the MMP 25 polypeptide and/or providing the subject with compounds that will modulate the level of the MMP25 production at the transcription and/or translation levels. The treatment can also be accomplished by administering MMP25 polypeptide or its DNA sequence in a form so as to effect production of MMP 25's activity in vivo.

2. To identify agonists and antagonists using the materials provided by the invention.

3. Treating conditions associated with MMP25 activity imbalance with the MMP polypeptides, antibodies or identified compounds.

V. DISCLOSURE OF THE INVENTION:

1. WITH RESPECT TO THIS INVENTION:

A.	HAVE ANY DISCUSSIONS OR OTHER CONTACTS BEEN MADE WITH POTENTIAL PURCHASERS?	YES___NO X
B.	HAS IT BEEN SOLD OR OFFERED FOR SALE?	YES___NO X
C.	IF IT PERTAINS TO A PROCESS, HAVE ANY STEPS BEEN TAKEN TO EMPLOY THE PROCESS COMMERCIALLY?	YES___NO X
D.	HAS IT BEEN DESCRIBED IN A PRINTED PUBLICATION?	YES___NO X
E.	HAS IT BEEN DISCLOSED IN A TALK OR A PAPER PRESENTED AT A PUBLIC MEETING?	YES___NO X
F.	HAS IT BEEN OTHERWISE DISCLOSED TO VENDORS OR CUSTOMERS?	YES___NO X
G.	IF NOT, IS ANY SUCH USE, SALE, PUBLICATION OR DISCLOSURE NOW CONTEMPLATED?	YES___NO X
H.	HAS IT BEEN REDUCED TO PRACTICE (I.E., MADE, CARRIED OUT, OR BUILT AND TESTED) OR HAS A MODEL OR PROTOTYPE BEEN BUILT?	YES___NO X
I.	IS THERE ANY AGREEMENT THAT DEALS WITH RIGHTS IN THIS INVENTION OR OWNERSHIP OF THIS INVENTION?	YES___NO X

2. IF ANY ANSWER TO ANY PART OF QUESTION 1 IS "YES," PLEASE INDICATE EARLIEST DATES,AND GIVE THE SURROUNDING CIRCUMSTANCES:

VI. DESCRIPTION:

PLEASE ADD ANY ADDITIONAL SHEETS AND DRAWINGS NECESSARY TO DESCRIBE THE INVENTION.

1. SUMMARY OF THE INVENTION:

The invention relates to a newly identified polynucleotide and polypeptide encoded and to the use of such polynucleotide and polypeptide and to their production. More particularly, the polynucleotide and polypeptide of the present invention is a novel member of the matrix metalloproteinase family, hereinafter referred to as MMP25 (See attached description).

2. WHAT PROBLEM IS SOLVED?

MMPs have been implicated in various pathological conditions such arthritis, atherosclerosis, aneurysm and tumor metastasis. Disease progression can be modulated by directly affecting the activity of MMPs. MMP25 can be used in MMP inhibitor selectivity studies to generate MMP family member specific inhibitors to reduce potential side effects. Further, MMP25 is a novel family member with unique properties and expression..

3. DESCRIBE ANY SIMILAR SYSTEM(S) OR METHOD(S) THAT YOU ARE AWARE OF AND THE ADVANTAGES OF THE INVENTION OVER THESE OTHER SYSTEM(S) O R METHOD(S).

4. TECHNICAL DESCRIPTION OF THE INVENTION (REFER TO DRAWINGS WITH REFERENCE NUMERALS).

See attached figures.

5. WHAT ASPECTS OF THE INVENTION CAN BE VARIED OR ALTERED, AND YET STILL ACCOMPLISH THE END RESULT OR OBJECT OF THE INVENTION?

6. SUPPORTING DOCUMENTATION AS TO WHEN AND WHERE THE INVENTION WAS FIRST CONCEIVED. PLEASE LIST ANY RELEVANT WRITTEN OR PICTORIAL MATERIAL (NOTEBOOK NUMBER AND PAGE, FILE REPORTS OR DRAWINGS, ETC.).

The database searches and EST sequence assembling were performed

The 3' RACE and sequence assembling were done
The 5' RACE and sequence assembling were done
The full-length sequence was obtained
The expression was done

WITNESS:	PRELIMINARY DISCLOSURE EVALUATED BY:
INVENTION WITNESSED AND UNDERSTOOD BY:	
SIGNATURE:	SIGNATURE:
PRINTED NAME: FRED RAMSDELL	PRINTED NAME:
TITLE: DIRECTOR, DISCOVERY BIOLOGY	TITLE:
DATE:	DATE:

MMP 25

Matrix metalloproteinase (MMP) gen family members have been implicated in a variety of physiological and pathological processes involving the degradation and remodeling of the extracellular matrix. Through EST database searches and various experimental methods, we have identified, cloned and characterized a novel MMP, MMP25.

i) The gene

A novel matrix metalloproteinase, MMP 25, was identified from EST database searches using the tBLASTN search algorithm with the conserved peptide sequences LVAAHELGHXLGLXHSXXXXAXMXXXY and HGDXXPFDGXXXXLAHAFXPGXGXGGDXHPDXDEXWT. The conserved MMP peptide sequences were obtained by aligning the protein sequences of MMP family members using a multiple sequence alignment program. After obtaining and assembling the ESTs which may encode putative MMPs, a novel MMP with 835 bp of assembled consensus sequence was obtained. Database searches clearly indicate it is a novel member of the MMP gene family.

To obtain full-length cDNA sequence cDNA library screening and RACE reactions were preformed. The final assembled MMP 25 cDNA sequence is 1832 bp in length with a coding region of 1539 bp (Figure 1). A polyadenylation sequence (ATTAAA) is located 24 bp upstream from the poly (A) sequence. The MMP 25 polypeptide is 513 amino acid residues in length. The amino acid sequence for MMP 25 contains a number of characteristic domains of the metalloproteinase gene family. These include a signal peptide, a pro-peptide, a zinc-dependent metalloproteinase domain and a hemopexin domain. Like other MMPs, the cystein-switch sequence (PCGVPD) is clearly located in the pro-peptide region of the MMP 25 (Figure 1 and 2). Despite the

conservation of various protein domains, the sequence similarity to other MMP family member is low. The highest overall sequence similarity (46% at amino acid level) is to members of the stromelysin subfamily which includes MMP3, MMP10 and MMP11.

ii) A shorter splice variant

A smaller splice variant for the MMP25 sequence has been identified by cDNA library screening and RACE reactions. The shorter version of the MMP 25 lacks the second Zinc binding region in the protease domain (Figure 2) which is required for the MMP activity. This suggests that the shorter splice variant probably encodes a non-functional polypeptide.

iii) The expression

The expression level of the MMP 25 gene was examined using RT-PCR on a tissue panel containing cDNA samples from 36 different normal tissues. Low levels of MMP25 gene expression could be seen in fetal skin and mammary gland at 35 cycles of amplification (Figure 3). The smaller splice variant is clearly visible in both tissues however, the functional implication of this shorter form of MMP25 is unclear.

iv). The chromosomal location

Using the G3 radiation hybrid panel with MMP25 gene specific primers (DMO 7560 CCGCAGAGAAGTAATGTTCTTTAAA and DMO 8563 TGATATCATAATAGATCCTCCATAGGTGCC), we have mapped MMP 25 to the

chromosome 11q22 region. Several other MMPs, including MMP1, MMP3, MMP7, MMP8, MMP10, MMP12, and MMP13 have also been mapped to this region.

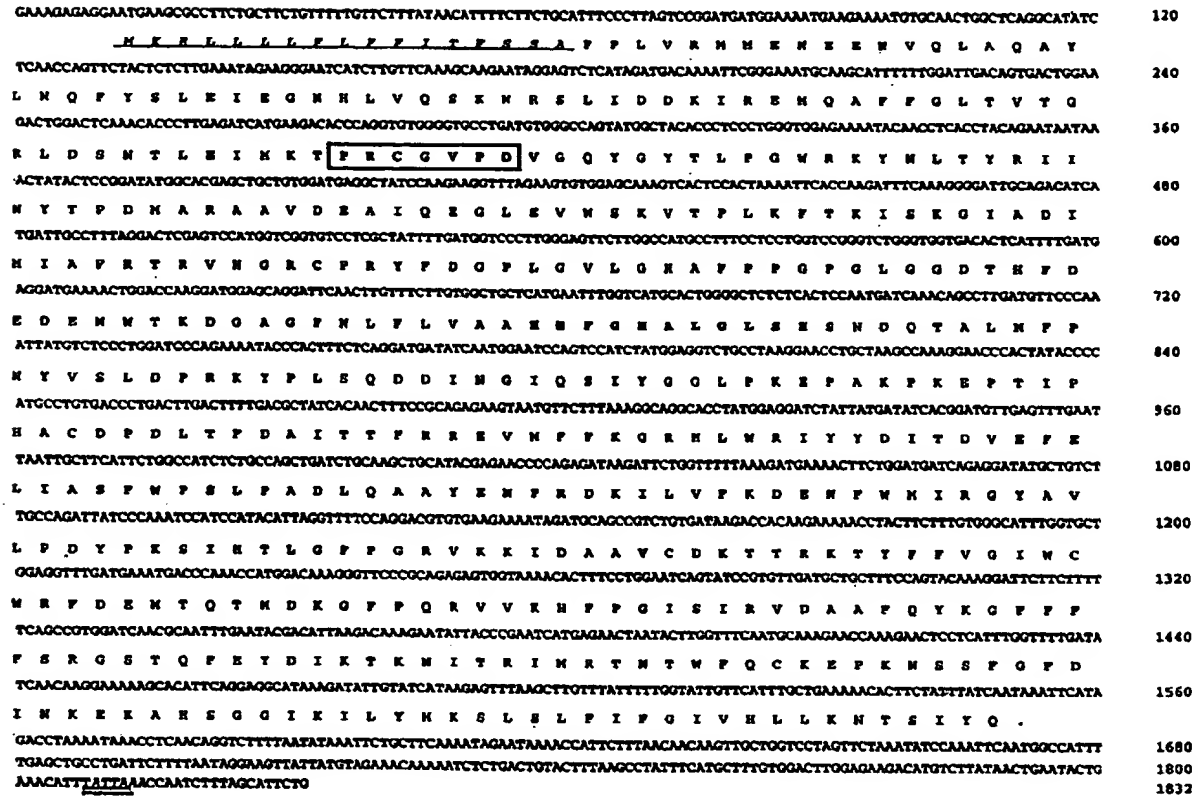

Figure 1 *Nucleotide and deduced amino acid sequences of MMP25. The putative signal peptide sequence is underlined and the cystein switch region sequence is boxed. The Zn binding region sequence is listed in bold face characters. The polyadenylation signal is listed in italic and underlined.*

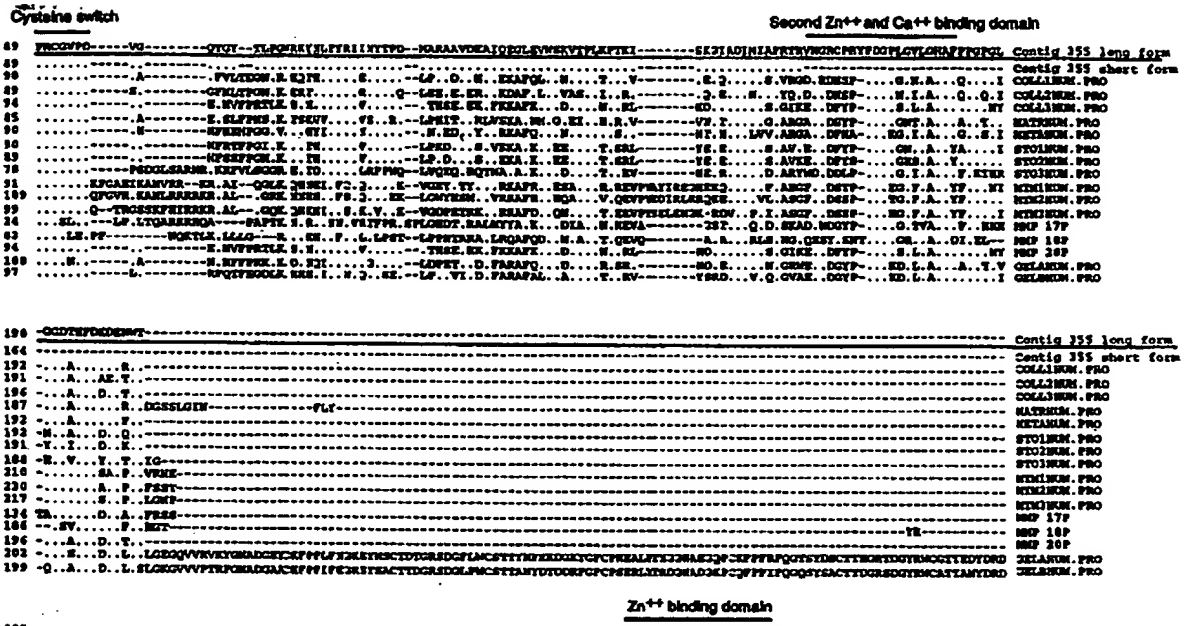

Figure 2 Alignment of the deduced amino acid sequence of the protease domain of contig 355 with other MMP gene family members. Gaps "-" have been introduced to maximize sequence homology. Amino acid sequences that match the sequence of mmp255 were replaced with dots "."

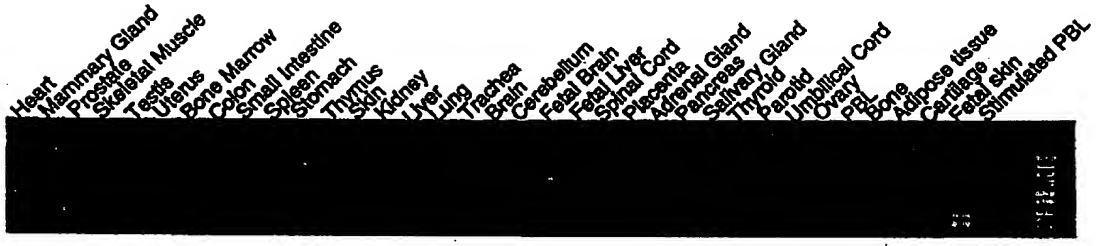

Figure 3 *Expression analysis of theMMP25 with RT-PCR using cDNA extracted from 36 normal tissues. Thirty-five cycles of amplifications were preformed usingMMP25 specific primer pair. Tissues used in the amplifications are indicated on top of each lane.*